

Use of the microalga *Scenedesmus obliquus* to remove cadmium cations from aqueous solutions

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Abstract The ability of a wild strain of *Scenedesmus obliquus*, isolated from a heavy metal-contaminated environment, to remove Cd^{2+} from aqueous solutions was studied at several initial concentrations. Viable biomass removed metal to a maximum extent of $11.4 \text{ mg}_{\text{Cd}}/\text{g}$ at $1 \text{ mg}_{\text{Cd}}/\text{l}$, with most Cd^{2+} being adsorbed onto the cell surface. A commercially available strain (ACOI 598) of the same microalga species was also exposed to the same Cd concentrations, and similar results were obtained for the maximum extent of metal removal. Heat-inactivated cells removed a maximum of $6.04 \text{ mg}_{\text{Cd}}/\text{g}$ at $0.5 \text{ mg}_{\text{Cd}}/\text{l}$. The highest extent of metal removal, analyzed at various pH values, was $0.09 \text{ mg}_{\text{Cd}}/\text{g}$ at pH 7.0. Both strains of the microalga tested have proven effective in removing a toxic heavy metal from aqueous solutions, hence supporting their choice for bioremediation strategies of industrial effluents.

Introduction

Contamination of water bodies by heavy metals leached from industrial effluents is currently a serious environmental problem (Bayramoğlu and Arica 2008; Fraile et al. 2005). This form of pollution has the particular disadvantage of not being susceptible to biodegradation, hence leading to bioaccumulation throughout the food chain (Doshi et al. 2007a).

Cadmium is one good example of such metal pollution. It is often present in paint pigments, alloys, metal platings and batteries (Pérez-Rama et al. 2002; Solisio et al. 2008). Being a non-essential metal to living organisms, it can displace essential metals (e.g. Zn) with specific biological functions, so chronic exposure to high levels of Cd may result in kidney or liver damage, bone degeneration, and even cancer (Doshi et al. 2007a; Solisio et al. 2008).

Several authors have been searching for alternative and better performing remediation strategies pertaining to toxic heavy metals, because conventional physico-chemical methods (e.g. precipitation and ion-exchange) are not fully effective; in addition, they are rather expensive (Bayramoğlu and Arica 2008; Doshi et al. 2007b), especially when the metal levels are of the ppm order of magnitude (Gupta and Rastogi 2008a; Yu and Kaewsarn 1999).

A more feasible approach relies on the metal binding and uptake capacities of living materials, which include microalgae in particular (Doshi et al. 2007b; Fraile et al. 2005; Leborans and Novillo 1996; Rollemberg et al. 1999; Solisio et al. 2008). Application of microbial biomass to remove toxic heavy metals has become relatively popular, owing to its high adsorbing capacity and low cost (Bayramoğlu and Arica 2008; Doshi et al. 2007b). Additionally, metals removed by adsorption onto the cell surface, may be successfully recovered, after desorption brought about by chemical agents: Costa and França (1998) reported that a 10.0 g/l EDTA solution could totally recover the Cd previously removed by adsorption onto the cell walls of the microalga *Tetraselmis chuii*, whereas Gupta and Rastogi (2008b) obtained 85 and 80% recoveries of Cd ions from

Oedogonium sp. biomass, when using HCl or EDTA as desorbing agents, respectively.

The level of metal removal by a microalga depends on several processing factors, such as supernatant metal and biomass concentrations, as well as pH and contact time (Aksu and Dönmez 2006; Solisio et al. 2008; Tang et al. 2002). Such a phenomenon occurs via a dual mechanism, which encompasses biosorption and bioaccumulation. The former is a passive event, which is independent of cell metabolism, and is based on physicochemical interactions between metal and functional groups on the cell wall. Conversely, bioaccumulation depends on cell metabolism, and takes place when metal ions are incorporated intracellularly (Özer et al. 1999; Rangsayatorn et al. 2002).

The aim of this study was to test the ability of a wild strain of *Scenedesmus obliquus*, isolated from a polluted site in Portugal, to remove Cd ions from aqueous solution—using either viable or inactivated biomass, upon exposure to several Cd concentrations and pH values. It has been claimed that microalgal species isolated from polluted environments are usually more resistant and more capable of accumulating heavy metals (Chong et al. 2000), so a reference strain of *S. obliquus* (ACOI 598) was also tested in this study, to ascertain whether the extent of metal removal under regular operating conditions was affected by strain source.

Experimental

Microalgal biomass and stock solution

The *Scenedesmus obliquus* strain (L) was isolated from a region of Northern Portugal that has been polluted with heavy metals for several decades—“Esteiro de Estarreja”. The contaminated sediments include mainly the following heavy metals: 835 mg Pb/kg, 66 mg Hg/kg and 3,620 mg Zn/kg, with other metals detected at lower levels (Oliveira et al. 2001). The *S. obliquus* strain (ACOI 598) was obtained from the alga culture collection (ACOI) held by University of Coimbra (Portugal).

Both microalga strains were cultivated in PHM medium (Borowitzka and Borowitzka 1988), with 1 g/l Tris–HCl buffer but without EDTA, and were maintained at 25°C under continuous illumination. Cultures in the exponential growth phase were used in all experimental batch cultures.

A stock solution of Cd^{2+} was prepared by diluting solid CdCl_2 in deionized water. Defined volumes of the stock solution were added to the growth medium, in order to obtain the desired final concentrations.

All material used was previously rinsed once with nitric acid, and several times with deionised water afterwards, to prevent analytical interferences.

Cd removal by viable biomass

Both strains of *S. obliquus* (strains L and ACOI 598) were exposed, in triplicate, at an initial biomass concentration of 0.02 g/l, to 0.05, 0.1, 0.25, 0.5 and 1 $\text{mg}_{\text{Cd}}/\text{l}$ for 7 days. Aliquots of 75 ml were collected daily, in duplicate, and were used to quantify biomass growth and Cd removal, both by adsorption on the cell wall and by absorption into the microalgal cells.

Cell growth was determined by measuring the optical density (OD) at 600 nm, and subsequently converting it to dry weight (DW) using a calibration curve prepared in advance. Metal removal was determined following the method of Matsunaga et al. (1999) and Pérez-Rama et al. (2002), after sample centrifugation at 4,000 rpm for 15 min at 4°C (to separate the biomass). Subsequently, the pellet was washed for 20 min with a 0.02 M EDTA solution (to remove Cd ions adsorbed onto the cell surface); this fraction was disposed of, and after another centrifugation, the pellet was digested with 1 ml of 15 M HNO_3 and 0.5 ml of 70% HClO_4 ; the Cd concentration in the supernatant was finally determined by atomic absorption spectrophotometry.

The total concentration of Cd removed by microalgal cells was calculated as the difference between the initial and the remaining Cd in the supernatant; the concentration of Cd adsorbed onto the cell surface was, in turn, determined as the difference between the total concentration of Cd removed and that of intracellular Cd.

Replicated blank controls, containing culture medium plus metal at each concentration tested, were considered; the Cd concentration remained stable in those flasks for the time frame of each experiment, so no redox reaction or adsorption onto the vessel walls took place to any measurable extent. The initial Cd concentrations in the experimental flasks were also confirmed by taking an aliquot of the culture medium before adding the microalga biomass, and assaying for Cd as described above.

Cd removal by inactive biomass

The biomass of *S. obliquus* strain (L) was harvested in the exponential phase, and inactivated by heating at 100°C for 24 h. Removal experiments using different initial concentrations (0.05, 0.1, 0.25, 0.5 and 1 $\text{mg}_{\text{Cd}}/\text{l}$), at an initial biomass concentration of 0.02 g/l, were conducted in triplicate. Following centrifugation, the supernatant was assayed for the remaining metal concentration.

Cd removal as affected by pH

The influence of pH was tested by exposing 0.27 g/l of viable biomass of *S. obliquus* strain (L), in triplicate, to an initial concentration of 0.3 $\text{mg}_{\text{Cd}}/\text{l}$, under pH values

ranging from 3.0 to 7.0. Aliquots were taken by 120 min and, after centrifugation, the supernatant was analysed for total Cd removed. In this test, the biomass concentration used was ca. 10 times higher than that of the remaining experiments, due to the reduced contact time.

Statistical analyses

Statistical analyses of the experimental data were conducted using SPSS, v. 16.0 (Chicago IL, USA). Analysis of variance (ANOVA) was applied to the experimental results, as well as Student's *t*-test and Tukey's test, aiming

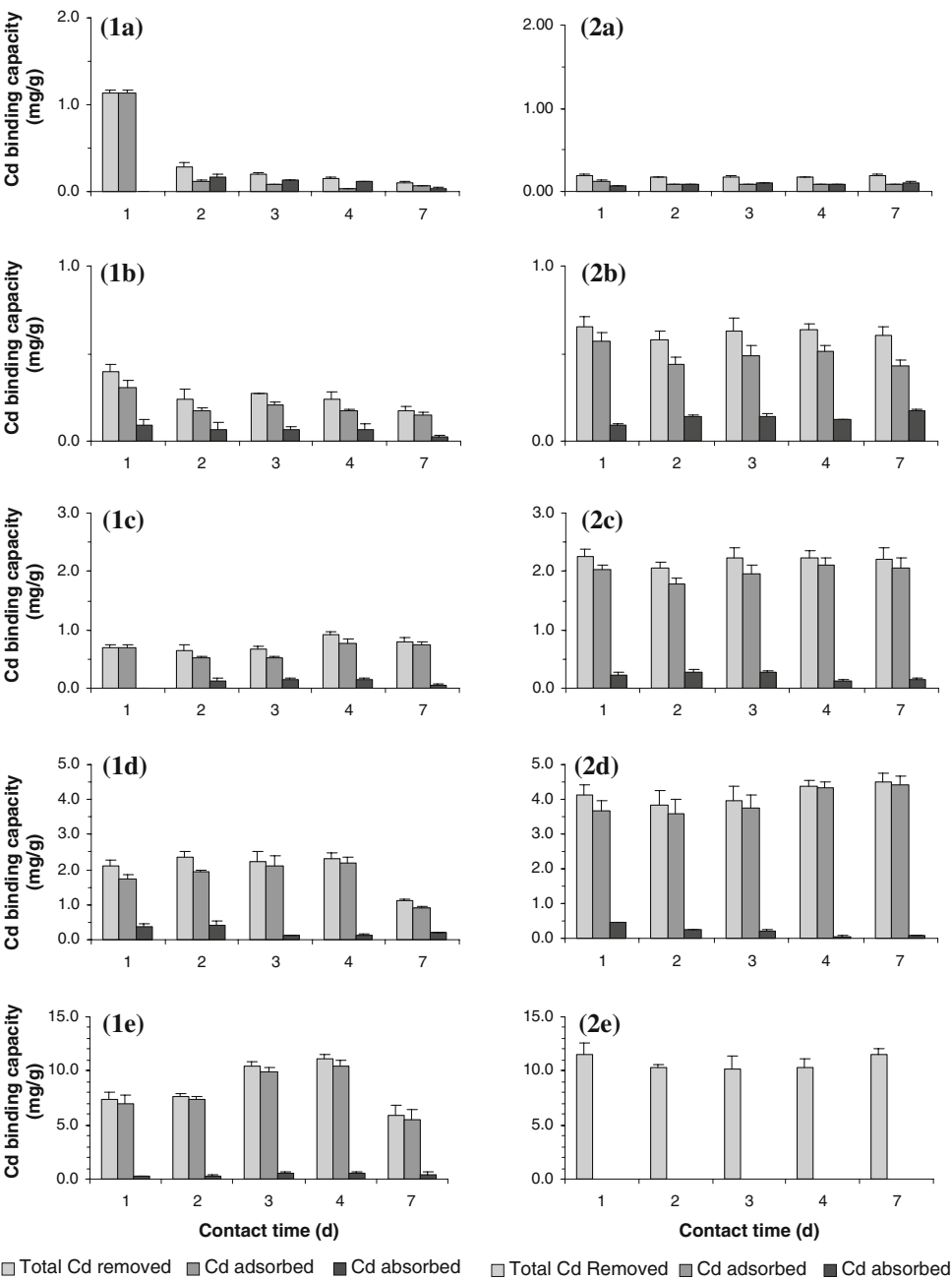
at pinpointing statistically significant (at the 5% level) differences between means.

Results

Cd removal by *S. obliquus* biomass

The degree of Cd removal by living cells of both strains, under various initial Cd concentrations, for a period of 7 days, is represented in Fig. 1. For both ecotypes, removal increased with increasing initial metal concentration, but

Fig. 1 Total amounts of Cd removed, adsorbed and absorbed, as a function of contact time (mean \pm standard deviation, $n = 3$), by viable (1) *Scenedesmus obliquus* (L) and (2) *S. obliquus* (ACOI 598) biomass, at various initial Cd concentrations, viz. **a** 0.05, **b** 0.1, **c** 0.25, **d** 0.5 and **e** 1 mg/l. The microalga biomass concentration was 0.02 g/l; the amounts of Cd adsorbed on and absorbed by the *S. obliquus* (ACOI 598) cells could not be measured at 1 mg/l



almost all metal biosorption was achieved within the first day of exposure. The maximum levels of removal were 11.4 and 11.5 mg/g, by *S. obliquus* strains (L) and (ACOI 598), respectively. Additionally (except for the lowest Cd concentration tested), the metal was removed mainly by adsorption onto the cell surface.

When inactivated biomass was employed, the Cd removal by *S. obliquus* strain (L), upon exposure for 90 min to the various initial metal concentrations, is depicted in Fig. 2; it is apparent that the total amount of Cd removed was higher at higher initial concentrations, with a maximum degree of removal of 6.04 mg_{Cd}/g at 0.5 mg_{Cd}/l. The maximum extent of Cd removal was achieved by 15 min, at all initial concentrations tested, and was followed by a slight decrease until an apparent equilibrium was eventually reached by 120 min.

Cd removal as affected by pH

The influence of solution pH upon the degree of Cd removal by the wild microalga biomass is represented in Table 1, for a period of 120 min. At higher pH, the total Cd

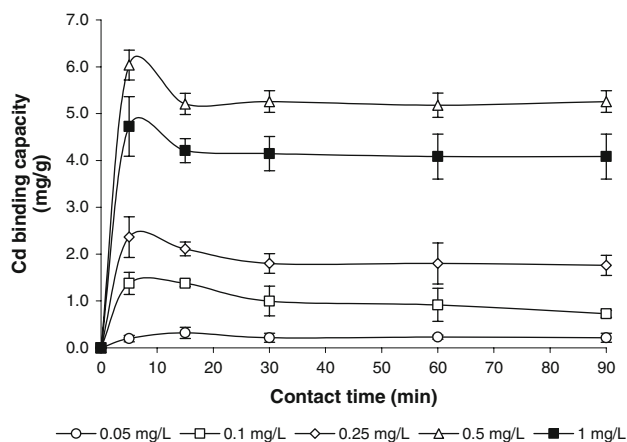


Fig. 2 Total amount of Cd removed, as a function of contact time (mean \pm standard deviation, $n = 3$), by inactivated *Scenedesmus obliquus* (L) biomass, at various initial Cd concentrations, viz. 0.05, 0.1, 0.25, 0.5 and 1 mg/l. The microalga biomass concentration was 0.02 g/l

Table 1 Total amount of Cd removed, as a function of pH (mean \pm standard deviation, $n = 3$), by viable *Scenedesmus obliquus* (L) biomass, by 120 min of exposure

pH	Concentration of Cd removed (mg/g)
3	0.036 \pm 0.007
4	0.018 \pm 0.008
5	0.054 \pm 0.007
6	0.034 \pm 0.004
7	0.087 \pm 0.011

The microalga biomass concentration was 0.27 g/l

removed was higher; a maximum removal extent of 0.087 mg_{Cd}/g was achieved at pH 7.0, which is significantly ($P < 0.05$) higher than that observed at the other pH values. The lowest removal extent (0.018 mg_{Cd}/g) was observed at pH 4.0, yet no significant ($P > 0.05$) differences were detected between this value and those obtained at either pH 3.0 or 6.0.

Discussion

Cd removal by *S. obliquus* biomass

Living microalga biomass has been used for bioremediation processes of heavy metal-contaminated wastewaters, owing to its ability to remove such contaminants, either by adsorption onto the cell surface or by incorporation into the cells themselves. Two distinct biochemical paths can thus be followed: biosorption (or adsorption of metal ions onto the cell surface) and bioaccumulation (or absorption of metal into the cell) (Rangsayatorn et al. 2002). In our study, the pattern of Cd removal—with maxima reached very early during exposure, followed by a slight decrease and eventually by a plateau, was consistent with that described by Torres et al. (1998). Additionally, the increase observed in total metal removal, as the metal concentrations in the supernatant solution were set higher, was also described elsewhere (Costa and França 1998; Özer et al. 1999). Except for the lowest Cd concentration (for which the amount of Cd uptake by the cells overcame the amount adsorbed), the metal was removed by both ecotypes chiefly by adsorption onto the cell surface. The cell wall of microorganisms has been claimed to play a crucial role as a defence mechanism—in that it is the first barrier to the uptake of toxic metals (Özer et al. 1999; Rangsayatorn et al. 2002). Maximum extents of Cd removal attained in our case were 11.4–11.5 mg_{Cd}/g, upon exposure to 1 mg_{Cd}/l. A similar finding was reported by Costa and França (1998): *T. chuii* was able to remove a maximum of 8.54 mg_{Cd}/g, when in the presence of 1 mg_{Cd}/l. Dönmez et al. (1999) reported, for other metals, a pattern identical to that obtained by us; when studying the biosorption capacity of the microalga *S. obliquus* upon exposure to Cu(II), Ni and Cr(VI), at concentrations ranging in 25–224 mg/l, they observed that maximum metal removal was attained for the highest initial metal concentration, viz. 26.8 mg/g for Cu(II), 24.7 mg/g for Ni and 30.2 mg/g for Cr(VI). Cain et al. (1980) also described that bioaccumulation of Cd by the same microalga increased with increasing external Cd concentrations, and claimed a maximum bioaccumulation of 3,031 ppm when subject to an initial concentration of 1.0 mg/l. Drbal et al. (1985) and Travieso et al. (1999) concluded that *S. obliquus* and

(immobilized) *Scenedesmus acutus* could remove a maximum of 39 and 74% of Cd from solution, respectively, following contact with an initial concentration of 0.5 mg/l of that metal. Furthermore, higher removal capacities were found by Terry and Stone (2002) in the case of *Scenedesmus abundans*, ranging from 139 to 574 mg/g, when the biomass was exposed to initial Cd concentrations from 5 to 20 mg/l.

Although it has been claimed that microalgae isolated from contaminated environments may exhibit a higher metal removal capacity than those grown in otherwise clean environments (Chong et al. 2000), this was not the case in our study. This realisation may be rationalized by the fact that Cd is not a major environmental pollutant of the site from which the strain was isolated, unlike other heavy metals, viz. Pb, Hg and Zn (Oliveira et al. 2001).

Inactivated microalgal biomass has been successfully used in heavy metal adsorption trials, for which it possesses the advantages of not requiring nutrients to survive, and withstanding highly toxic environments (Aksu and Dönmez 2006; Solisio et al. 2008). The fast binding of Cd within the first minutes of contact—which is essentially followed by a plateau, hence suggests that the metal is removed via interactions with functional groups on the surface; Bayramoğlu and Arica (2008) have concluded likewise. Comparing the Cd removal capacity of viable and inactivated biomass, via a Student's *t*-test, it is clear that the latter removes significantly ($P < 0.05$) more Cd ions when exposed to 0.1, 0.25 and 0.5 mg_{Cd}/l. As reported by Bayramoğlu and Arica (2008) when studying metal removal by heat-inactivated fungi, thermal treatment may generate additional binding sites via denaturation of proteins on the cell wall structures, thus promoting metal removal.

Cd removal as affected by pH

The extent of metal removal using biosorbents depends on the pH of the supernatant solution, as this parameter affects the protonation state of the functional groups on the biomass cell wall (Bayramoğlu and Arica 2008; Gupta and Rastogi 2008a). At low pH, the surface charge of the cell wall is indeed positive, so it constrains binding of (positive) metal cations. As pH is raised, more ligands bearing negative charges become exposed on the surface of the cell wall, with subsequent attraction of metal ions (Gupta and Rastogi 2008a; Tüzün et al. 2005). Our data indicated that the optimum pH for Cd removal is 7.0 (which was the highest pH tested); therefore, an increase in pH enhances metal removal from solution, as there is a lower competition between protons and metal cations for the active sites on the biomass cell wall. Several other researchers have shown a similar dependence of metal removal on pH: e.g. Tüzün et al. (2005)

and Bayramoğlu and Arica (2008) described an increase in Cd removal up to pH 6.0, whereas Fraile et al. (2005) reported maximum Cd removal at pH 8.0.

Conclusions

The microalga *S. obliquus* is well suited to remove Cd²⁺ ions from aqueous solutions, and such a removal is a rapid process; the good sorption capacity of those microorganisms is comparable to that of other microalgae described in the literature. On the other hand, the commercial strain presents a Cd removal capacity essentially similar to that of the wild strain.

Most metal is removed from solution via adsorption onto the cell surface (except at the lowest Cd concentration tested, for which absorption appears to dominate). Accordingly, heat-inactivated biomass proved able to remove most Cd²⁺ ions from solution, thus confirming its potential applicability in wastewater treatment.

Finally, Cd removal from solution depends on pH, with a maximum level of removal at ca. pH 7.0.

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References

- Aksu Z, Dönmez G (2006) Binary biosorption of cadmium(II) and nickel(II) onto dried *Chlorella vulgaris*: co-ion effect on mono-component isotherm parameters. *Process Biochem* 41:860–868. doi:10.1016/j.procbio.2005.10.025
- Bayramoğlu G, Arica MY (2008) Removal of heavy mercury(II), cadmium(II) and zinc(II) metal ions by live and heat inactivated *Lentinus edodes* pellets. *Chem Eng J* 143:133–140. doi:10.1016/j.cej.2008.01.002
- Borowitzka MA, Borowitzka LJ (1988) Algal media and sources of algal cultures. In: Borowitzka MA, Borowitzka LJ (eds) *Microalgal biotechnology*. Cambridge University Press, Cambridge, pp 456–466
- Cain JR, Paschal DC, Hayden CM (1980) Toxicity and bioaccumulation of cadmium in the colonial green alga *Scenedesmus obliquus*. *Arch Environ Contam Toxicol* 9:9–16. doi:10.1007/BF01055495
- Chong AMY, Wong YS, Tam NFY (2000) Performance of different microalgal species in removing nickel and zinc from industrial wastewater. *Chemosphere* 41:251–257. doi:10.1016/S0045-6535(99)00418-X
- Costa ACA, França FP (1998) The behaviour of the microalgae *Tetraselmis chuii* in cadmium-contaminated solutions. *Aquacult Int* 6:57–66. doi:10.1023/A:1009221820135
- Dönmez GÇ, Aksu Z, Öztürk A, Kutsal T (1999) A comparative study on heavy metal biosorption characteristics of some algae. *Process Biochem* 34:885–892. doi:10.1016/S0032-9592(99)00005-9

- Doshi H, Ray A, Kothari IL (2007a) Biosorption of cadmium by live and dead *Spirulina*: IR spectroscopic, kinetics and SEM studies. *Curr Microbiol* 54:213–218. doi:[10.1007/s00284-006-0340-y](https://doi.org/10.1007/s00284-006-0340-y)
- Doshi H, Ray A, Kothari IL (2007b) Bioremediation potential of live and dead *Spirulina*: spectroscopic, kinetics and SEM studies. *Biotechnol Bioeng* 96:1051–1063. doi:[10.1002/bit.21190](https://doi.org/10.1002/bit.21190)
- Drbal K, Véber K, Zahradník J (1985) Toxicity and accumulation of copper and cadmium in the alga *Scenedesmus obliquus* LH. *Bull Environ Contam Toxicol* 34:904–908. doi:[10.1007/BF01609824](https://doi.org/10.1007/BF01609824)
- Fraile A, Penche S, González F, Blázquez ML, Muñoz JA, Ballester A (2005) Biosorption of copper, zinc, cadmium and nickel by *Chlorella vulgaris*. *Chem Ecol* 21:61–75. doi:[10.1080/02757540512331334933](https://doi.org/10.1080/02757540512331334933)
- Gupta VK, Rastogi A (2008a) Biosorption of lead from aqueous solutions by green algae *Spirogyra* species: kinetics and equilibrium studies. *J Hazard Mater* 152:407–414. doi:[10.1016/j.jhazmat.2007.07.028](https://doi.org/10.1016/j.jhazmat.2007.07.028)
- Gupta VK, Rastogi A (2008b) Equilibrium and kinetic modelling of cadmium (II) biosorption by nonliving algal biomass *Oedogonium* sp. from aqueous phase. *J Hazard Mater* 153:759–766. doi:[10.1016/j.jhazmat.2007.09.021](https://doi.org/10.1016/j.jhazmat.2007.09.021)
- Leborans GF, Novillo A (1996) Toxicity and bioaccumulation of cadmium in *Olithodiscus luteus* (Raphidophyceae). *Water Res* 30:57–62. doi:[10.1016/0043-1354\(95\)00084-X](https://doi.org/10.1016/0043-1354(95)00084-X)
- Matsunaga T, Takeyama H, Nakao T, Yamazawa A (1999) Screening of marine microalgae for bioremediation of cadmium-polluted seawater. *J Biotechnol* 70:33–38. doi:[10.1016/S0168-1656\(99\)00055-3](https://doi.org/10.1016/S0168-1656(99)00055-3)
- Oliveira RS, Dodd JC, Castro PML (2001) The mycorrhizal status of *Phragmites australis* in several polluted soils and sediments of an industrialised region of Northern Portugal. *Mycorrhiza* 10:241–247. doi:[10.1007/s005720000087](https://doi.org/10.1007/s005720000087)
- Özer A, Özer D, Dursun G, Bulak S (1999) Cadmium (II) adsorption on *Cladophora crispata* in batch stirred reactors in series. *Waste Manag* 19:233–240. doi:[10.1016/S0956-053X\(99\)00082-3](https://doi.org/10.1016/S0956-053X(99)00082-3)
- Pérez-Rama M, Alonso JA, López CH, Vaamonde ET (2002) Cadmium removal by living cells of the marine microalga *Tetraselmis suecica*. *Bioresour Technol* 84:265–270. doi:[10.1016/S0960-8524\(02\)00045-7](https://doi.org/10.1016/S0960-8524(02)00045-7)
- Rangsayatorn N, Upatham ES, Kruatrachue M, Pokethitiyook P, Lanza GR (2002) Phytoremediation potential of *Spirulina* (Arthrospira) *platensis*: biosorption and toxicity studies of cadmium. *Environ Pollut* 119:45–53. doi:[10.1016/S0269-7491\(01\)00324-4](https://doi.org/10.1016/S0269-7491(01)00324-4)
- Rollemberg MC, Gonçalves MLSS, Santos MMC, Botelho MJ (1999) Thermodynamics of uptake of cadmium by *Chlorella marina*. *Bioelectrochem Bioenerg* 48:61–68. doi:[10.1016/S0302-4598\(98\)00220-7](https://doi.org/10.1016/S0302-4598(98)00220-7)
- Solisio C, Lodi A, Soletto D, Converti A (2008) Cadmium biosorption on *Spirulina platensis* biomass. *Bioresour Technol* 99:5933–5937. doi:[10.1016/j.biortech.2007.11.002](https://doi.org/10.1016/j.biortech.2007.11.002)
- Tang YZ, Gin KYH, Aziz MA (2002) Equilibrium model for cadmium adsorption by green algae in a batch reactor. *J Environ Eng* 128:304–312. doi:[10.1061/\(ASCE\)0733-9372\(2002\)128:4\(304\)](https://doi.org/10.1061/(ASCE)0733-9372(2002)128:4(304))
- Terry PA, Stone W (2002) Biosorption of cadmium and copper contaminated water by *Scenedesmus abundans*. *Chemosphere* 47:249–255. doi:[10.1016/S0045-6535\(01\)00303-4](https://doi.org/10.1016/S0045-6535(01)00303-4)
- Torres E, Cid A, Herrero C, Abalde J (1998) Removal of cadmium ions by the marine diatom *Phaeodactylum tricornutum* Bohlin accumulation and long-term kinetics of uptake. *Bioresour Technol* 63:213–220. doi:[10.1016/S0960-8524\(97\)00143-0](https://doi.org/10.1016/S0960-8524(97)00143-0)
- Travieso L, Cañizares O, Borja R, Benítez F, Domínguez AR, Dupeyrón R, Valiente V (1999) Heavy metal removal by microalgae. *Bull Environ Contam Toxicol* 62:144–151. doi:[10.1007/s001289900853](https://doi.org/10.1007/s001289900853)
- Tüzün İ, Bayramoğlu G, Yalçın E, Başaran G, Çelik G, Arica MY (2005) Equilibrium and kinetic studies on biosorption of Hg(II), Cd(II) and Pb(II) ions onto microalgae *Chlamydomonas reinhardtii*. *J Environ Manag* 77:85–92. doi:[10.1016/j.jenvman.2005.01.028](https://doi.org/10.1016/j.jenvman.2005.01.028)
- Yu Q, Kaewsarn P (1999) A model for pH dependent equilibrium of heavy metal biosorption. *Korean J Chem Eng* 16:753–757. doi:[10.1007/BF02698347](https://doi.org/10.1007/BF02698347)